

AMENDMENTS TO THE SPECIFICATION

Please insert the accompanying Sequence Listing (pages 1-109).

Please amend the paragraph at page 14, line 6 through page 15, line 4, as follows:

Figures 1A, 1B, and 1C show an HPLC analysis of the D-NorFES-A protease indicator (F^1 -Asp-Ala-Ile-Pro-Nle-Ser-Ile-Pro-Cys- F^2 , SEQ ID NO:1) where F^1 is a donor (D) fluorophore (5'-carboxytetramethylrhodamine (C2211) and F^2 is an acceptor (A) fluorophore (rhodamine X acetamide (R492))) before and after the addition of elastase. Fig. 1A: HPLC before the addition of elastase showing the late eluting peak representing the intact indicator molecule. Fig. 1B: HPLC after the addition of elastase with detection at 550 nm where both fluorophores absorb. Fig. 1C HPLC after the addition of elastase with detection at 580 nm where F^2 absorbs maximally.

Please amend the paragraph at page 15 line 17 through page 16, line 7, as follows:

Figure 5 illustrates fluorescence of a DEVD, a DEVN, and an ICE substrate. To one hundred μ l of assay buffer (50mM HEPES buffer pH 7.5, 10% (w/v) sucrose and 0.1% (w/v) CHAPS) containing 1 μ M of substrate DEVD (compound 2 of Example 8), DEVN (compound 3 of Example 8) and ICE (compound 5 of Example 8) 10 μ l of Jurkat cell lysate was added and incubated for 16 hours at 37°C. The Jurkat cells' lysate was prepared from the cells that had been stimulated by antiFas antibody at 1 μ g/ml concentration for 6 hours. The fluorescence intensity for the substrate solution alone is indicated in Figure 5 as a horizontal lined bar marked as t = 0 hr and the fluorescence intensity of the lysate and substrate solution mixture after 16 hr is indicated by vertical line bar and is marked as t = 16 hr digestion. 10 μ l cell lysate was pre-incubated with 50 μ m ZVAD-FMK (benzyloxycarbonyl valanyl alanyl aspartyl-fluoromethylketone) at 37°C for 30 min. then added to the substrate solution. The fluorescence intensity after 16 hours for this mixture is indicated by the bar marked as ZVAD-FMK (inhibitor). Lastly, pre-incubated cell lysate with iodoacetamide(alkylating agent for sulphydryl group) and PMSF (for inhibiting serine proteases) was added to the substrate solution. The fluorescence intensity after 16 hours at 37°C is indicated by bar marked as Iodoacetamide/PMSF. The DEVN substrate is a negative control substrate where the P1, Asp, residue is replaced by Asn. The CPP32 protease requires the P1 residue to be aspartic acid residue. The four bar graphs for the DEVN substrate (Fig. 5) clearly indicate that the activated cell lysate do not contain any other protease that digest the DEVD substrate, since the intensity for 16 hour digestion is the same as the substrate alone.

The bar graphs for the DEVD substrate indicate that the activate cell lysate do contain CPP32 protease and this protease activities are inhibited by ZVAD-FMK, known CPP32 protease inhibitor. The contribution of any other proteases in digesting DEVD substrate is very small as indicated by the difference between the intensities of ZVAD-FMK bar to Iodoacetamide/PMSF bar.

Please amend the paragraph at page 21, lines 1-8, as follows:

In certain preferred embodiments, the protease binding region of the protease indicators of the present invention is selected to be symmetric about the cleavage site. Thus, for example, where a binding region is:

Ile-Pro-Met-Ser-Ile (SEQ ID NO:2)

(e.g. α -1 anti-trypsin) and the cleavage occurs between Met and Ser then a four amino acid residue binding region based on this sequence would be:

**- P₂ -P₁ - P₁' - P₂' -
-Pro-Met-Ser-Ile- (SEQ ID NO:3)**

Please amend the paragraph at page 21, lines 14-21, as follows:

Various amino acid substitutions may be made to the amino acids comprising the protease binding domain to increase binding specificity, to eliminate reactive side chains, or to reduce the conformational entropy (decrease degrees of freedom) of the molecule. Thus, for example, it is often desirable to substitute methionine (Met) residues, which bear a oxidizable sulfur, with norleucine. Thus, in the example given, a preferred protease binding region will have the sequence:

**- P₂ - P₁ - P₁' -P₂' -
-Pro-Nle-Ser-Ile- (Seq ID NO:4)**

Please amend Table 2 at pages 23-25, as follows:

Table 2. Illustration of the design of the conformation determining regions and protease binding site based on known protease substrate and inhibitor sequences. Italics indicate residues that are added to create a bend and to increase rigidity of the conformation determining regions. Normal font indicates

residues of the substrate or inhibitor that forms the protease binding site. The thick line indicates the location at which the protease binding site is cleaved.

Substrate/Inhibitor	CDR (C ¹)			Protease Binding Site (P) P ₂			CDR (C ²)		<u>SEQ ID NO</u>	
	C ¹ ₅	C ¹ ₄	C ¹ ₃	P ₁	P _{1'}	P ₂	C ² ₃	C ² ₄		
α-1 anti-trypsin	Asp	Ala	Ile	Pro	Met <i>Nle</i>	Ser	Ile	Pro <i>Aib</i>	Cys Lys	<u>5</u>
plasminogen activator inhibitor 2	Asp	Met <i>Aib</i> Pro	Thr <i>Aib</i> <i>Pro</i>	Gly	Arg	Thr	Gly	Pro <i>Aib</i>	Cys Lys	<u>6</u>
neutrophil leukocyte elastase inhibitor	Asp	Ala <i>Aib</i> <i>Pro</i>	Thr <i>Aib</i> <i>Pro</i>	Phe	Cys	Met <i>Nle</i>	Leu	Pro <i>Aib</i>	Cys Lys	<u>7</u>
anti-plasmin inhibitor	Asp	<i>Aib</i>	Ser <i>Aib</i> <i>Pro</i>	Arg	Met <i>Nle</i>	Ser	Leu	Pro <i>Aib</i>	Cys Lys	<u>8</u>
anti α-1 thrombin	Asp	Ile <i>Aib</i> <i>Pro</i>	Ala <i>Aib</i> <i>Pro</i>	Gly	Arg	Ser	Leu	Pro <i>Aib</i>	Cys Lys	<u>9</u>
α-1 antichymotrypsin	Asp	<i>Aib</i>	Thr <i>Aib</i> <i>Pro</i>	Leu	Leu	Ser	Leu	Pro <i>Aib</i>	Cys Lys	<u>10</u>
interstitial type III (human liver) collagen	Asp	Gly <i>Aib</i>	Pro <i>Aib</i>	Leu	Gly	Ile	Ala	Pro <i>Aib</i>	Cys Lys	<u>11</u>
type I collagen for collagenase Bovine α 1	Asp	Gly <i>Aib</i> <i>Pro</i>	Pro <i>Aib</i>	Gln	Gly	Ile	Leu	Pro <i>Aib</i>	Cys Lys	<u>12</u>
type I collagen chick α2	Asp	Gly <i>Aib</i> <i>Pro</i>	Pro <i>Aib</i>	Gln	Gly	Leu	Leu	Pro <i>Aib</i>	Cys Lys	<u>13</u>
human α1 type II collagen	Asp	Gly <i>Aib</i> <i>Pro</i>	Pro <i>Aib</i>	Gln	Gly	Ile	Ala	Pro <i>Aib</i>	Cys Lys	<u>14</u>
type III collagen - AIA	Asp	Gly <i>Aib</i> <i>Pro</i>	Pro <i>Aib</i>	Gln	Ala	Ile	Ala	Pro <i>Aib</i>	Cys Lys	<u>15</u>
type III collagen (human skin)	Asp	Gly <i>Aib</i> <i>Pro</i>	Pro <i>Aib</i>	Gln	Gly	Ile	Ala	Pro <i>Aib</i>	Cys Lys	<u>16</u>
human α 2 macroglobulin	Asp	Gly <i>Aib</i> <i>Pro</i>	Pro <i>Aib</i>	Glu	Gly	Leu	Arg	Pro <i>Aib</i>	Cys Lys	<u>17</u>
stromelysin cleavage sites of stromelysin-1d	Asp	Asp <i>Aib</i> <i>Pro</i>	Val <i>Aib</i> <i>Pro</i>	Gly	His	Phe	Arg	Pro <i>Aib</i>	Cys Lys	<u>18</u>
stromelysin cleavage sites of stromelysin-1	Asp	Asp <i>Aib</i> <i>Pro</i>	Thr <i>Aib</i> <i>Pro</i>	Leu	Glu	Val	Met <i>Nle</i>	Pro <i>Aib</i>	Cys Lys	<u>19</u>
stromelysin cleavage site	Asp	Arg	Ala	Ile	His	Ile	Gln	Pro	Cys	<u>20</u>

of proteoglycan link protein		<i>Aib Pro</i>	<i>Aib Pro</i>					<i>Aib</i>	Lys	
gelatinase type IV collagenase site of 72 K gelatinases	Asp	Asp <i>Aib Pro</i>	Val <i>Aib Pro</i>	Ala	Asn	Tyr	Asn	<i>Pro Aib</i>	Cys Lys	<u>21</u>
gelatinase type IV cleavage of gelatin	Asp	Gly <i>Aib Pro</i>	Pro <i>Aib</i>	Ala	Gly	Glu	Arg	<i>Pro Aib</i>	Cys Lys	<u>22</u>
gelatinase type IV cleavage of gelatin	Asp	Gly <i>Aib Pro</i>	Pro <i>Aib</i>	Ala	Gly	Phe	Ala	<i>Pro Aib</i>	Cys Lys	<u>23</u>
type III collagen (human skin)	Asp	Gly <i>Aib Pro</i>	Pro <i>Aib</i>	Gln	Gly	Leu	Ala	<i>Pro Aib</i>	Cys Lys	<u>24</u>
Human FIB-CL propeptide	Asp	Asp <i>Aib Pro</i>	Val <i>Aib Pro</i>	Ala	Gln	Phe	Val	<i>Pro Aib</i>	Cys Lys	<u>25</u>
Cathepsin D (Thyroglobulin Fragment Tg1)	Asp	Asp <i>Aib Pro</i>	Gly Pro <i>Aib</i>	His	Phe	Leu	Arg	<i>Pro Aib</i>	Cys Lys	<u>26</u>
Cathepsin D (Thyroglobulin Fragment Tg2)	Asp	Thr <i>Aib Pro</i>	Thr Pro <i>Aib</i>	Glu	Leu	Phe	Ser	<i>Pro Aib</i>	Cys Lys	<u>27</u>
Cathepsin D (Thyroglobulin Fragment Tg3)	Asp	Lys <i>Aib Pro</i>	Phe Pro <i>Aib</i>	Ile	Ala	Phe	Leu	<i>Pro Aib</i>	Cys Lys	<u>28</u>
Cathepsin D (Thyroglobulin Fragment Tg4)	Asp	Phe <i>Aib Pro</i>	Ser Pro <i>Aib</i>	His	Phe	Val	Arg	<i>Pro Aib</i>	Cys Lys	<u>29</u>
Prostate Specific Antigen (PSA) (Seminolgelin, Sg) Sg1	Asp	Gln <i>Aib Pro</i>	Gln Pro <i>Aib</i>	Leu	Leu	His	Asn	<i>Pro Aib</i>	Cys Lys	<u>30</u>
Prostate Specific Antigen (PSA) (Seminolgelin, Sg) Sg2	Asp	Ser <i>Aib Pro</i>	Ile Pro <i>Aib</i>	Gln	Tyr	Thr	Tyr	<i>Pro Aib</i>	Cys Lys	<u>31</u>
Prostate Specific Antigen (PSA) (Seminolgelin, Sg) Sg3	Asp	Ser <i>Aib Pro</i>	Ser Pro <i>Aib</i>	Gln	Tyr	Ser	Asn	<i>Pro Aib</i>	Cys Lys	<u>32</u>
Prostate Specific Antigen (PSA) (Seminolgelin, Sg) Sg4	Asp	Ser <i>Aib Pro</i>	Ser Pro <i>Aib</i>	Ile	Tyr	Ser	Gln	<i>Pro Aib</i>	Cys Lys	<u>33</u>
Gelatin α 1 (type 1)	Asp	Gly <i>Aib Pro</i>	Pro <i>Aib</i>	Ala	Gly	Val	Gln	<i>Pro Aib</i>	Cys Lys	<u>34</u>

Please amend the paragraph at page 26, lines 21-34, as follows:

In a particularly preferred embodiment, aa¹ and aa¹⁰ are independently selected from the group consisting of lysine, ornithine and cysteine; aa², aa³, aa⁸ and aa⁹ are independently selected from the group consisting of an amino acid or a dipeptide consisting of Asp, Glu, Lys, Ornithine, Arg,

Citulline, homocitrulline, Ser, homoserine, Thr, and Tyr; aa⁵, aa⁴, aa⁶, and aa⁷ are independently selected from the group consisting of proline, 3,4-dehydroproline, hydroxyproline, alpha aminoisobutyric acid and N-methyl alanine; X is selected from the group consisting of Gly, βAla, γAbu, Gly-Gly, Ahx, βAla- Gly, βAla-βAla, γAbu-Gly, βAla-γAbu, Gly-Gly-Gly, γAbu-γAbu, Ahx-Gly, βAla-Gly-Gly, Ahx-βAla, βAla-βAla-Gly, Gly-Gly-Gly-Gly (SEQ ID NO:35), Ahx-γAbu, βAla-βAla-βAla, γAbu-βAla-Gly, γAbu-γAbu-Gly, Ahx-Ahx, γAbu-γAbu-βAla, and Ahx-Ahx-Gly; Y is selected from the group consisting of Gly, βAla, γAbu, Gly-Gly, Ahx, Gly-βAla, βAla-βAla, Gly-γAbu, γAbu-βAla, Gly-Gly-Gly, γAbu-γAbu, Gly-Ahx, Gly-Gly-βAla, βAla-Ahx, Gly-βAla-βAla, Gly-Gly-Gly-Gly(SEQ ID NO:35), γAbu-Ahx, βAla-βAla-βAla, Gly-βAla-γAbu, Gly-γAbu-γAbu, Ahx-Ahx, βAla-γAbu-γAbu, and Gly-Ahx-Ahx.

Please amend Table 3 on page 28 as follows:

Table 3. Illustration of the design of the conformation determining regions and protease binding sites in molecules having P domains larger than 4 amino acids. The P1 residue is underlined. Z is benzyloxycarbonyl group, K[TFA] means Lys(N(epsilon)trifluoroacetyl), Fm is Fmoc (preferably attached to the alpha amino group of the amino terminal residue e.g., Lysine (K)). O indicates tetrahydroisoquinoline-3-carboxylic acid. Aib, designated as B, can be replaced by Pro.

Name	aa ¹	aa ² - aa ³	aa ⁴	aa ⁵	X	P	Y	aa ⁶	aa ⁷	aa ⁸ - aa ⁹	aa ¹⁰	S ²	<u>SEQ ID NO</u>	
PAI-2	K	D		B		T <u>GRTG</u>		P				K	GY	<u>36</u>
PAI-2(b)	K	D	P	P		TGRTG		P	P			K	GY	<u>37</u>
DEVD	K	D		B		DEV <u>DGID</u>		P				K	GY	<u>38</u>
DevN	K	D		B		DEV <u>NGID</u>		P				K	GY	<u>39</u>
PARP	K	D		B		E <u>VDGID</u>		P				K	GY	<u>40</u>
ICE	K	DY		B		A <u>DGID</u>		P				K	GY	<u>41</u>
Fm-DEVD	Fm-K	D		B		DE <u>VDGID</u>		P				K	GY	<u>42</u>
Fm-DEVN	Fm-K	D		B		DE <u>VNGID</u>		P				K	GY	<u>43</u>
Fm-PARP	Fm-K	D		B		E <u>VDGID</u>		P				K	GY	<u>44</u>
Fm-KNFES	Fm-K	D		-		AIP <u>MSI</u>		P				K	GY	<u>45</u>
	Fm-K	D				AIP <u>NluSI</u>		P				K	GY	<u>46</u>
Fm-G2D2D	Fm-K	D		B		G <u>DEV<u>DGID</u></u>	G	P				K	GY	<u>47</u>

Fm-CGD2D	Fm-K	D		B	J	GDEV <u>D</u> GID	G J	P			K	GY	<u>48</u>
Z-CGD2D	Z-K	D		B	J	GDEV <u>D</u> GID	G J	P			K	GY	<u>49</u>
Fm-ICE	Fm-K	DY		B		ADGID		P			K	GY	<u>50</u>

Please amend Table 4, at pages 29-35, as follows:

Table 4. Illustration of the design of the conformation determining regions and protease binding sites in molecules having P domains larger than 4 amino acids. The P1 residue is underlined. Z is benzyloxycarbonyl group, K[TFA] means Lys(N(epsilon)trifluoroacetyl), Fm is Fmoc (preferably attached to the alpha amino group of the amino terminal residue *e.g.*, Lysine (K). O indicates tetrahydroisoquinoline-3-carboxylic acid. Aib, designated as B, can be replaced by Pro. J is a C

Substrate class	aa ¹	aa ² -aa ³	aa ⁴	aa ⁵	X	P	Y	aa ⁶	aa ⁷	aa ⁸ -aa ⁹	aa ¹⁰	S ²	SEQ ID NO
CPP32	Fa-K	D		P	JG	DEVD <u>G</u> IN	GJ	P			K	GY	<u>51</u>
CPP32	Fm-K	D		P	JG	DEVD <u>G</u> IN	GJ	P			K amid e		<u>52</u>
CPP32	Fm-K	D		P	JG	(d-O)DE <u>V</u> GIN	GJ	P			K	GY	<u>53</u>
CPP32	Fm-K	D		P	JG	DEVD <u>G</u> IN	G	P			K	GY	<u>54</u>
CPP32	Fm-K	D		P	G	DEVD <u>G</u> IN	GJ	P			K	GY	<u>55</u>
CPP32	Fm-K	D		P	JG	DEVD <u>G</u> ID	GJ	P			K amid e		<u>56</u>
CPP32	Fm-K	D		P	JG	EEVE <u>G</u> IN	GJ	P			K	GY	<u>57</u>
CPP32	Fm-K	D		P	JG	D(dF)V <u>D</u> GIN	GJ	P			K	GY	<u>58</u>
CPP32	Fm-K	D		P	JG	(d-D)EV <u>(d-D)</u> GIN	GJ	P			K	GY	<u>59</u>
CPP32	Fm-K	D		P	JG	DEVD <u>G</u> IN	GJ	P			K	GY	<u>60</u>
CPP32	Fm-K	DB			JG	DEV <u>N</u> GIN	GJ	P			K	GY	<u>61</u>
CPP32	Fm-K	DB			JG	DEVD <u>G</u> ID	GJ	P			K	GY	<u>62</u>
CPP32	Fm-K	DB			JG	DEVD <u>G</u> IN	GJ	P			K	GY	<u>63</u>
CPP32	Fm-K	DB			JG	DEV <u>N</u> GID	GJ	P			K	GY	<u>64</u>

CPP32	K	D		B	JJ	GDEV <u>D</u> GI	JJ	P			K	GY	<u>65</u>
CPP32	K	D		B	J	GNEV <u>D</u> GI	GJ	P			K	GY	<u>66</u>
CPP32	K	D		B	J	GDEV <u>N</u> GI	GJ	P			K	GY	<u>67</u>
CPP32	K	D		B	J	GNEV <u>N</u> GI	GJ	P			K	GY	<u>68</u>
CPP32	K	D		B	J	GDEV <u>N</u> GI	GJ	P			K	GY	<u>69</u>
CPP32	K	D		B	J	GNEV <u>N</u> GI	GJ	P			K	GY	<u>70</u>
CPP32	K	D		B	JG	ODEV <u>D</u> GI	GJ	P			K	GK	<u>71</u>
CPP32	K	D		B	JG	dODEVD <u>GID</u>	GJ	P			K	GY	<u>72</u>
CPP32	K	D		B	JG	WDEV <u>DG</u> ID	GJ	P			K	GY	<u>73</u>
CPP32	K	D		B	JG	dWDEV <u>DGID</u>	GJ	P			K	GY	<u>74</u>
CPP32	K	D		B	JG	dOdODEV <u>DGID</u>	GJ	P			K	GY	<u>75</u>
CPP32	K	D		B	JG	dWdWDE <u>VDGID</u>	GJ	P			K	GY	<u>76</u>
CPP32	K	D		B		YVAD <u>GI</u> D		P			K	GY	<u>77</u>
CPP32	K	D		B		YVAD <u>GI</u> N		P			K	GY	<u>78</u>
CPP32	K	D		B		YVAN <u>GI</u> N		P			K	GY	<u>79</u>
CPP32	K	D		B	G	YVAD <u>GI</u> D	G	P			K	GY	<u>80</u>
CPP32	K	D		B	G	YVAD <u>GI</u> N	G	P			K	GY	<u>81</u>
CPP32	K	D		B	G	YVAN <u>GI</u> N	G	P			K	GY	<u>82</u>
CPP32	K	D		B	JG	YVAD <u>GI</u> D	GJ	P			K	GY	<u>83</u>
CPP32	K	D		B	JG	YVAN <u>GI</u> D	GJ	P			K	GY	<u>84</u>
CPP32	K	D		B	JG	YVAN <u>GI</u> N	GJ	P			K	GY	<u>85</u>
CPP32	K	D		B	JG	YVAD <u>GI</u> N	GJ	P			K	GY	<u>86</u>
CPP32	K	D		B	JG	dYVAD <u>GI</u> N	GJ	P			K	GY	<u>87</u>
CPP32	K	D		B		YVHDAP <u>V</u>		P			K	GY	<u>88</u>
CPP32	K	D		B		YVHDAP <u>V</u>		P			K	GY	<u>89</u>
CPP32	K	D		B		YVHDAP <u>V</u>		P			K	GY	<u>90</u>

CPP32	K	D		B	G	YVHDAP V	G	P			K	GY	<u>91</u>
CPP32	K	D		B	G	YVHDAP V	G	P			K	GY	<u>92</u>
CPP32	K	D		B	G	YVHDAP V	G	P			K	GY	<u>93</u>
CPP32	K	D		B	JG	YVHDAP V	GJ	P			K	GY	<u>94</u>
CPP32	K	D		B	JG	YVHDAP V	GJ	P			K	GY	<u>95</u>
CPP32	K	D		B	JG	YVHDAP V	GJ	P			K	GY	<u>96</u>
CPP32	K	D		B	JG	YVHDAP V	GJ	P			K	GY	<u>97</u>
CPP32	K	D		B	JG	YVHDAP V	GJ	P			K	GY	<u>98</u>
CPP32	K	D		B	JG	dYVHDA PV	GJ	P			K	GY	<u>99</u>
Lamin-A	Fm-K	D		P	JG	LVEIDNG	J	P			K	GY	<u>100</u>
Lamin-A	FM-K	DP			JG	LVEIENG	J	P			K	GY	<u>101</u>
Lamin-A	K	D		B		LVEIDNG		P			K	GY	<u>102</u>
Lamin-A	K	D		B	G	LVEIDNG	G	P			K	GY	<u>103</u>
Lamin-A	K	D		B	JG	LVEIDNG	GJ	P			K	GY	<u>104</u>
Lamin-A	K	D		B	JG	LVEINNG	GJ	P			K	GY	<u>105</u>
ProCPP32A sp175	Fm-K	D		P	J	GIETESG V	GJ	P			K	GY	<u>106</u>
ProCPP32A sp175	Fm-K	D		P	J	GIETDSG	J	P			K	GY	<u>107</u>
ProCPP32A sp175	Fm-K	D		P	J	GIETESG	J	P			K	GY	<u>108</u>
ProCPP32A sp175	K	D		B		GIETDSG VDD		P			K	GY	<u>109</u>
ProCPP32A sp175	K	D		B		GIETNSG VDD		P			K	GY	<u>110</u>
ProCPP32A sp175	K	D		B	G	GIETDSG VDD	G	P			K	GY	<u>111</u>
ProCPP32A sp175	K	D		B	G	GIETNSG V	G	P			K	GY	<u>112</u>
ProCPP32A sp175	K	D		B	J	GIETDSG V	J	P			K	GY	<u>113</u>
ProCPP32A sp175	K	D		B	J	GIETNSG V	J	P			K	GY	<u>114</u>
ProCPP32A sp175	K	D		B	JG	GIETDSG V	GJ	P			K	GY	<u>115</u>
ProCPP32A sp175	K	D		B	JG	GIETNSG V	GJ	P			K	GY	<u>116</u>
ProCPP32A sp28	K	D		B		GSESMD SGISLD		P			K	GY	<u>117</u>

ProCPP32A sp28	K	D		B	G	GSESMD SG	G	P			K	GY	<u>118</u>
ProCPP32A sp28	K	D		B	JG	GSESMD SG	GJ	P			K	GY	<u>119</u>
NS3 NS5A/5B	K	D		B	JG	DVVCCS MS	GJ	P			K	GY	<u>120</u>
NS3 NS5A/5B	K	D		B	JG	DVVCDMS	GJ	P			K	GY	<u>121</u>
NS3 NS5A/5B	K	D		B	JG	DVVCCSdMS	GJ	P			K	GY	<u>122</u>
NS3 NS5A/5B	K	D		B	JG	DVVCDMS	GJ	P			K	GY	<u>123</u>
NS3 NS5A/5B	K	D		B	JG	DVVCCPdMS	GJ	P			K	GY	<u>124</u>
NS3 NS5A/5B	K	D		B	JG	EDVVCCS	GJ	P			K	GY	<u>125</u>
NS3 NS5A/5B	K	D		B	JG	EDVVCDMS	GJ	P			K	GY	<u>126</u>
NS3 NS5A/5B	K	D		B	JG	EDdVVC CP	GJ	P			K	GY	<u>127</u>
NS3 NS5A/5B	K	D		B	JG	EDdVVC DP	GJ	P			K	GY	<u>128</u>
NS3 NS5A/5B	K	D		B	JG	DdVVCCS dMS	GJ	P			K	GY	<u>129</u>
NS3 NS5A/5B	K	D		B	JG	DVdVCD SdMS	GJ	P			K	GY	<u>130</u>
NS3 NS5A/5B	K	D		B	JG	DdVVCCP dMS	GJ	P			K	GY	<u>131</u>
NS3 NS5A/5B	K	D		B	JG	DVVCCS M	GJ	P			K	GY	<u>132</u>
NS3 NS5A/5B	K	D		B	JG	DVVCDMS	GJ	P			K	GY	<u>133</u>
NS3 NS5A/5B	K	D		B	JG	VCCSM	GJ	P			K	GY	<u>134</u>
NS3 NS5A/5B	K	D		B	JG	VCDSM	GJ	P			K	GY	<u>135</u>
NS3 NS4A/4B	K	D		B	JG	DEMEEC SQHL		P			K	GY	<u>136</u>
NS3 NS4A/4B	K	D		B	JG	DEMEEC PQHL		P			K	GY	<u>137</u>
NS3 NS4A/4B	K	D		B	JG	DEMEED SQHL		P			K	GY	<u>138</u>
NS3 NS4A/4B	K	D		B	JG	EMEECS QHL		P			K	GY	<u>139</u>
NS3 NS4A/4B	K	D		B	JG	EMEECP QHL		P			K	GY	<u>140</u>
NS3 NS4A/4B	K	D		B	JG	EMEEDS QHL		P			K	GY	<u>141</u>
NS3 NS4A/4B	K	D		B	JG	EMEECS QHL	G	P			K	GY	<u>142</u>
NS3 NS4A/4B	K	D		B	JG	EMEECP QHL	G	P			K	GY	<u>143</u>

NS3 NS4A/4B	K	D		B	JG	EMEEDS QHL	G	P			K	GY	<u>144</u>
NS3 NS4A/4B	K	D		B	JG	EMEE <u>S</u> QHL	GJ	P			K	GY	<u>145</u>
NS3 NS4A/4B	K	D		B	JG	EMEE <u>C</u> P QHL	GJ	P			K	GY	<u>146</u>
NS3 NS4A/4B	K	D		B	JG	EMEE <u>D</u> S QHL	GJ	P			K	GY	<u>147</u>
Ext. PAI-2	K	D		B	JG	VMTGRT G	J	P			K	GY	<u>148</u>
Ext. PAI-2	K	D		B	JG	VdMTGR TG	J	P			K	GY	<u>149</u>
Ext. PAI-2	K	D		B	JG	VMTGRT G	J	P			K	GY	<u>150</u>
Ext. PAI-2	K	D		B	JG	VMTGRT G	J	P			K	GY	<u>151</u>
Thromb	K	D		B	JG	VMTGR <u>G</u>	J	P			K	GY	<u>152</u>
Thromb	K	D		B	JG	VMTGR <u>G</u>	GJ	P			K	GY	<u>153</u>
Thromb	K	D		B	JG	VdmTGR G	GJ	P			K	GY	<u>154</u>
Urokinase	Fm-K	D		P	J	TGRT							<u>155</u>
Urokinase		Fm-D		P		TGRT	G	P			K	GY	<u>156</u>
Urokinase	Fm-K	D		P		VMTGRT	GJ	P			K	GY	<u>157</u>
Urokinase	Fm-K	D		P		TGRT	GJ	P			K	GY	<u>158</u>
Urokinase	Fm-K	D		P	JG	TGRT	GJ	P			K	GY	<u>159</u>
Urokinase	Fm-K	D		P	JG	TGRT	G	P			K	GY	<u>160</u>
Urokinase	Fm-K	D		P	G	TGRT	G	P			K	GY	<u>161</u>
Urokinase	K	D		P	J	TGRTG	J	P			K	GY	<u>162</u>
Urokinase	K	D		P	C3	TGRTG		P			K	GY	<u>163</u>
Urokinase	K	D		P	C7	TGRTG		P			K	GY	<u>164</u>
Urokinase	K	D		B	JG	VMTGR <u>V</u> G	J	P			K	GY	<u>165</u>
Urokinase	K	D		B	JG	VdMTGR VG	J	P			K	GY	<u>166</u>
F12A	K	D		B	JG	VMTGRA G	J	P			K	GY	<u>167</u>
F12A	K	D		B	JG	VdMTGR AG	J	P			K	GY	<u>168</u>
Swedish KM/NL amyloid	Fm-K	D		P	JG	SEVKLD AEF GC5 PKGY	GJ	P			K	GY	<u>169</u>
Swedish KM/NL	Fm-K	D		P	JG	S(d-E)VK (d-L)DA	GJ	P			K	GY	<u>170</u>

amyloid						E(d-F)							
Swedish KM/NL amyloid	Fm-K	D		P	JG	S(d-E)VK (d-L)DA E(d-F)	GJ	P			K	GY	<u>171</u>
Swedish KM/NL amyloid	K	D		B	JG	SEVN <u>L</u> D AEF	GJ	P			K	DD Y	<u>172</u>
Swedish KM/NL amyloid	Fm-K	D		B	JG	SEVN <u>L</u> D AEF	GJ	P			K	DD Y	<u>173</u>
Swedish KM/NL amyloid	K	D		B	JG	SEVK <u>L</u> D AEF	GJ	P			K	DD Y	<u>174</u>
Native Amyloid	K	D		B	JG	SEVK <u>M</u> D AEF	GJ	P			K	DD Y	<u>175</u>
Cathepsin G	K	D		B	JG	SEVK <u>M</u> D DEF	GJ	P			K	DD Y	<u>176</u>
Cathepsin G	K	D		B	JG	SEVN <u>L</u> D DEF	GJ	P			K	DD Y	<u>177</u>
APP[709-710]	K	D		B	JG	GVVIATV IVIT	GJ	P			K	DD Y	<u>178</u>
APP[708-719]	K	D		B	JG	YGVVIAT VIVIT	GJ	P			K	DD Y	<u>179</u>
APP[711-716]	K	D		B	JG	VIATVI	GJ	P			K	DD Y	<u>180</u>
APP[708-713]	K	D		B	JB	YGVVIA	GJ	P			K	DD Y	<u>181</u>
PSA Sg1	K	D		B	JJ	Q <u>LL</u> H <u>N</u>	JJ	P			K		<u>182</u>
PSA Sg1	K	D		B	JG	Q <u>LL</u> H <u>N</u>	GJ	P			K		<u>183</u>
PSA Sg1	K	D		B	G	Q <u>LL</u> H <u>N</u>	G	P			K		<u>184</u>
PSA Sg1	K	D		B		Q <u>LL</u> H <u>N</u>		P			K		<u>185</u>
PSA Sg2	K	D		B	JJ	SI <u>QY</u> TY	JJ	P			K		<u>186</u>
PSA Sg2	K	D		B	JG	SI <u>QY</u> TY	GJ	P			K		<u>187</u>
PSA Sg2	K	D		B	G	SI <u>QY</u> TY	G	P			K		<u>188</u>
PSA Sg2	K	D		B		SI <u>QY</u> TY		P			K		<u>189</u>
PSA Sg3	K	D		B	JJ	SS <u>QY</u> SN	JJ	P			K		<u>190</u>
PSA Sg3	K	D		B	JG	SS <u>QY</u> SN	GJ	P			K		<u>191</u>
PSA Sg3	K	D		B	G	SS <u>QY</u> SN	G	P			K		<u>192</u>
PSA Sg3	K	D		B		SS <u>QY</u> SN		P			K		<u>193</u>
PSA Sg4	K	D		B	JJ	SSI <u>Y</u> S <u>Q</u>	JJ	P			K		<u>194</u>
PSA Sg4	K	D		B	JG	SSI <u>Y</u> S <u>Q</u>	GJ	P			K		<u>195</u>
PSA Sg4	K	D		B	G	SSI <u>Y</u> S <u>Q</u>	G	P			K		<u>196</u>
PSA Sg4	K	D		B		SSI <u>Y</u> S <u>Q</u>		P			K		<u>197</u>
Cathepsin D	Fm-K	D		P	JG	SEVNLD AEF	GJ	P			K	GY	<u>198</u>
Caspase-9	Fm-K	D		P	JG	LEHDGIN	GJ	P			K	GY	<u>199</u>
Caspase-8	Fm-	D		P	JG	LET <u>D</u> GIN	GJ	P			K	GY	<u>200</u>

	K												
Caspase-1	Fm-K	D		P	JG	WEHDGI N	GJ	P			K	GY	<u>201</u>
Caspase-1	Fm-K	D		P	JG	YVHDG	J	P			K	GY	<u>202</u>
Caspase-1	Fm-K	D		P	JG	YVHDGI N	GJ	P			K	GY	<u>203</u>
Caspase-1	Fm-K	D		P	JG	YVHDAP V	GJ	P			K	GY	<u>204</u>
Caspase-1	Fm-K	D		P	JG	YVHDAP V		P			K	GY	<u>205</u>
Caspase-1	Fm-K	D		P		YVHDAP V	GJ	P			K	GY	<u>206</u>
Caspase-1	Fm-K	D		P	JG	YVHDA		P			K	GY	<u>207</u>
Granzyme B	Fm-K	DP			JG	IEPDS	GJ	P			K	GY	<u>208</u>
Collagenase	Fm-K	DP			JG	PLGIAGI	GJ	P			K	GY	<u>209</u>
HIV-1 protease	Fm-K	DP			JG	SQNYPIV Q	GJ	P			K	GY	<u>210</u>
Hepatitis C protease	Fa-K	DP			JG	EDVVCC S	GJ	P			K	GY	<u>211</u>

Please amend the paragraph at page 59, lines 24-27, as follows:

The P₁' residue can be changed to introduce either charged amino acid side chains or a structurally rigid, *e.g.*, proline, residue as illustrated in the Table 3, substrate sequences for Hepatitis C viral protease substrate NS3 NS5A/5B of DVVCCSMS (SEQ ID NO:212, normal substrate) and DVVCCPdMS (SEQ ID NO:213, inhibitor). The underlined residues are the P₁ residues.

Please amend the paragraph at page 63, lines 8-11, as follows:

Fluorophores were linked to the amino terminus via the α -amino group of Aspartic acid residue (D) and to the ϵ -amino group of lysine (K). Labeling was accomplished by the displacement of a succinimidyl group linked to 6-TMR or DER. The structure of the peptide, called NorFES-KGY (SEQ ID NO:214) is:

Please amend the paragraph at page 65, lines 11-20, as follows:

In addition, (homodoubly-labeled) PAI-2, CS-1 (a 31 residue long peptide) and two DEVD-like peptides were synthesized and derivatized. PAI-2 and CS-1 allowed the dye-dye dimer formation. The CS-1 peptide showed that in a significantly longer peptide the dye-dye dimer structure

can be formed. Note this peptide contained four proline residues in the amino terminal side of the putative cleavage site Ile-Leu bond. There was one proline in the carboxyl domain as well. The results from the CS-1 peptide support a potentially larger sequence between the two dyes (fluorophores). Two DEVD-like peptide's amino acid sequences that did not allow the formation of productive H-type dimers are F₁-DEVDGIDPK[F₁]GY (SEQ ID NO:186215) and F₁-PDEVGDGIDPK[F₁]GY (SEQ ID NO:187216).

Please amend Table 12 at page 66, lines 1-5, as follows:

Table 12. Compounds assayed for cellular uptake. Abbreviations used in the following table are: F¹: carboxytetramethylrhodamine; Z: benzyloxycarbonyl group; Fm: Fmoc group; K[F1]: F¹ is covalently attached through the epsilon amino group of lysine (K). Single letter amino acid residues are used in the sequences except for Nlu for norleucine, B for aminoisobutyric acid and J for epsilon amino caproic acid residue. H: HPLC, FM: Fluorescence microscopy, FC: flow cytometry.

Structure	Cellular uptake/ magnitude	Uptake checked by	SEQ ID
			NO
1 Fm-K[F1] DAIPNluSIPK[F1]GY	Yes/high	FM	<u>188-217</u>
2 K[F1] DAIPNluSIPK[F1]GY	Yes/weak	FM	<u>189-218</u>
3 Fm-DAIPNluSIPK[F1]GY	No/	FM	<u>190-219</u>
4 Fm-K[F1]DBDEVGDGIDPK[F1]GY	Yes/high	FM & FC	<u>191-220</u>
5 K[F1]DBDEVGDGIDPK[F1]GY	Yes/weak	FM	<u>192-221</u>
6 Fm-K[F1]DBDEVNGIDPK[F1]GY	Yes/high	FM	<u>193-222</u>
7 K[F1]DBDEVNGIDPK[F1]GY	Yes/weak	FM & H	<u>194-223</u>
8 Fm-K[F1]DBEVGDGIDPK[F1]GY	Yes/high	FM & FC	<u>195-224</u>
9 K[F1]DYBADGIDPK[F1]GY	Yes/weak	FM	<u>196-225</u>
10 Fm-K[F1]DBGDEVGDGIDGPK[F1]GY	Yes/high	H & FC	<u>197-226</u>
11 Fm-K[F1]DBJGDEVGDGIDGJPK[F1]GY	Yes/high	FC	<u>198-227</u>
12 Z-K[F1]DBJGDEVGDGIDGJPK[F1]GY	Yes/weak	FM	<u>199-228</u>
13 Fm-K[F1]DYBADGIDPK[F1]GY	Yes/high	FM	<u>200-229</u>

14 K[F1]DBEVGDIDPK[F1]GY Yes/weak FM 201-230

Please amend the paragraph at page 67, lines 15-26, as follows:

The elastase substrate, Fm-K[F1]DAIPNluSIPK[F1]GY (SEQ ID NO:231), (where F1 was carboxytetramethylrhodamine, Fm was Fmoc, K[F1] was F1 covalently attached through the epsilon amino group of lysine (K), and Fm-K is the Fmoc group covalently attached at the alpha amino group of the amino terminal lysine residue) was used with HL-60 cells. Cells were incubated with various concentrations of elastase substrate ranging from 10 nM to 10 µM for 5 minutes to 60 minutes. Then the cells were diluted 5-fold with RPMI 1640 medium containing 5% serum or with phosphate buffered saline. The samples were centrifuged and washed once more with 1 ml of washing solution. After centrifugation and removal of the washing solution, cell pellets were loosened with about 25 ul of medium and these cells were transferred to a glass capillary. Capillary tubes were then placed on a glass microscope slide and examined under a fluorescence microscope using standard rhodamine filters.

Please amend the paragraph at page 68, lines 12-29, as follows:

Control cells without substrate incubation and the sample with the greatest expected fluorescence signals were used to set the instrument detector parameters. For example after 15 minutes incubation of Jurkat cells with substrate compound #11 Fm-CGD2D: Fm-K[F1]DBJGDEVGDIDGJPK[F1]GY (SEQ ID NO:4-232, where F1 was carboxytetramethylrhodamine; Fm was Fmoc, K[F1] was F1 covalently attached through the epsilon amino group of lysine (K), Nlu was norleucine, B was aminoisobutyric acid, and J was epsilon-aminocaproic acid) an increase of about 10 channels indicating cellular uptake of the substrates was measured. Note substrate #11 was not completely quenched. Hence, a small amount of background fluorescence would be expected from the intact substrate. Signals from the cells that had been activated with 1 µg/ml of anti-Fas antibody, CH11 clone for 1 to 6 hours indicated an increase in peak channel number. As much as a ten-fold increase in fluorescence intensity was observed. When the cells were co-incubated with the CPP32 protease inhibitor ZVAD-fluoromethylketone at 50 µM along with an apoptosis inducing agent, e.g., anti-Fas antibody, this observed increase in fluorescence intensity was eliminated. This indicated that the signal from compound 11 was due to the CPP32

protease activity which was inhibitable by ZVAD-FMK. Hence, the observed fluorescence intensity in each cell as determined by flow cytometric analysis served as a direct measure of the intracellular CPP32 protease activity.

Please amend the paragraph at page 69, lines 18-27, as follows:

Jurkat cells are normally grown in 10% fetal calf serum containing RPMI 1640, at 37°C in a 5% CO₂ atmosphere. When the serum content was dropped to 4%, the Jurkat cell growth rate not only slowed down but also a significant number of cells died within 36 hours. The cell density used was about 400,000 cell per ml. After 36 hours, control wells contained about 50% dead cells (trypan blue-positive cells), whereas the wells containing 0.1 or 1.0 μM concentration of compound #11 (Table 12) "Fm-CGD2D" or Fm-K[F1]DBJGDEVDGIDGJPK[F1]GY (SEQ ID NO:6 233) showed only 10% or 8% nonviable cells. Hence, compound #11 which exhibits efficient cellular uptake slowed down apoptosis in these Jurkat cells where it acted as a CPP32 protease inhibitor or a CPP32 activating protease inhibitor.

Please amend the paragraph at page 71, line 25 through page 72, line 19, as follows:

The parent compound Fm-DEVD has the following composition: Fmoc-K[F1]DBDEVGDIDPK[F1]GY (SEQ ID NO:7 234). The bold face underlined letters are the protease recognition sequence consisting of 7 amino acid residues. Compound #10 contains two glycine extensions at both ends of this protease recognition sequence. The central protease recognition domain now is 8 residues long GDEVGDID (SEQ ID NO:8 235), since the glycine residue at the amino terminus is a part of native sequence. The two glycine residues which are inherently more flexible than other amino acids, *e.g.*, alanine, provide less conformational constraint or, conversely, more flexibility than compound 4 (Table 12) and thereby permit greater flexion when combined with Aib or Pro residues. Additional insertion of amino caproic acid at both termini with five methylene groups in addition to the one present in glycine provides further relaxation of the constrained conformation and, thus, greater flexibility for the protease recognition domain, GDEVGDID (SEQ ID NO:9 236). This progression of flexibility resulted in an increased hydrolysis rate with the CPP32 protease since CPP32 recognizes a more flexible protease recognition domain than does elastase. Support for this statement is that the CPP32 protease cleavage site in the proform of its physiological substrate, poly(ADP-ribose) polymerase, PARP, is located between two well-folded domains. . In addition, the cleavage site within

the inactive proform of CPP32 which is a member of proteases family called caspases by another caspase family member protease, caspase-8, has been shown to localized between the two folded domains of CPP32 called p17 and p12. Hence, it is expected that such a protease cleavage site would not be rigidly held or its conformation would be expected to be less defined than the remaining molecule. In order to provide these structural features to the substrate, introduction of flexible residues such as glycine, epsilon amino caproic acid, beta alanine, and amino butyric acid would be expected to play important roles in regulating the backbone flexibility of the substrate's central protease recognition domain. These additional preferred residues for the conformation determining domain are also expected to provide the needed bend-inducing influence.

Please amend the paragraph at page 72, lines 26-32, as follows:

These examples provide a tetrapeptide and a pentapeptide comprising Lys-Asp-Aib-Gly (SEQ ID NO:237) or Lys-Asp-Aib-Ahx-Gly (SEQ ID NO:238) where Ahx is epsilon amino caproic acid (*i.e.* NH₂-(CH₂)₅-COOH). The fluorophore is attached to epsilon amino group of the lysine residue. The carboxyl terminal CDR domain is defined as a tripeptide Gly-Pro-Lys and a tetrapeptide Gly-Ahx-Pro-Lys (SEQ ID NO:239). The hydrolysis rate was increased by 3-fold between compounds 4 (Fm-DEVD: Fm-K[F1]DBDEVDGIDPK[F1]GY (SEQ ID NO:9 240)) and 10 (Fm-G2D2D: Fm-K[F1]DBGDEVDGIDGPK[F1]GY) (SEQ ID NO:10 241))

Please amend the paragraph at page 72, line 33 through page 73, line 3, as follows:

As illustrated in Figure 5, the hydrolysis rate was further increased by *ca.* 3-fold over the above glycine residue insertion with the amino caproic amino acid (Ahx) addition, compound 11 (Fm-CGD2D: Fm-K[F1]DB Ahx GDEVGDIDG Ahx PK[F1]GY, (SEQ ID NO:10 242)). Hence, overall at least a 9-fold increase in substrate hydrolysis rate was accomplished (compounds 4 and 11, Table 12).